ES 221 Problem Set 2

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1 Question 1: Water Solubility as a Function of pH of Candidate Antipsychotics

We first begin the drug delivery effort for a new atypical antipsychotic by predicting the solubility of four candidate tertiary amine compounds as a function of pH. These candidates have low solubility (S_0) at room temperature but are found to exhibit high binding affinity to both the dopamine D2 receptors and serotonin 5HT2A receptors. Given the four compounds $D2$, $D5$, $D6$, and $D12$, we quantify their solubility (S_w) using the formula:

$$S_w = S_o(1 + \frac{[H^+]}{K_a}) \tag{1}$$

The free base water solubility, S_0, and pK_a of the amine on each compound are reported as 11.2, 4.6, 2.9, and $5.8 \mu g/mL$, and as 8.6, 9.1, 9.3, and 8.6, respectively. The association constant, K_a, is related to the pK_a by the relation $pK_a = -log_{10}(K_a)$, or $K_a = 10^{-pK_a}$. A similar relation is used to find the proton concentraiton from the pH. Thus, we obtain corresponding solubilities at pH of 5, 6, and 7 as shown in Table 1. Note that these solubilities are given in mg/mL.

Table 1: Solubility of Candidate Compounds.

Compound	**pH = 5**	**pH = 6**	**pH = 7**
D2	44.6	4.47	0.457
D5	57.9	5.80	0.584
D6	57.9	5.80	0.582
D12	23.1	2.32	0.237

Based on these results, we can conclude that at a pH of 5, all four of the compounds exhibit a solubility larger than $10mg/mL$. The observed trend in solubility is consistent with expectations, i.e. that, as pH is reduced, the free base form of the amine is protonated and remains in solution as a solvated cation (below the limit set by the solubility product), allowing more of the free base to be dissolved and reducing the driving force for precipitation.

2 Question 2: IV Bolus Pharmacokinetic Analysis of Lead Compound

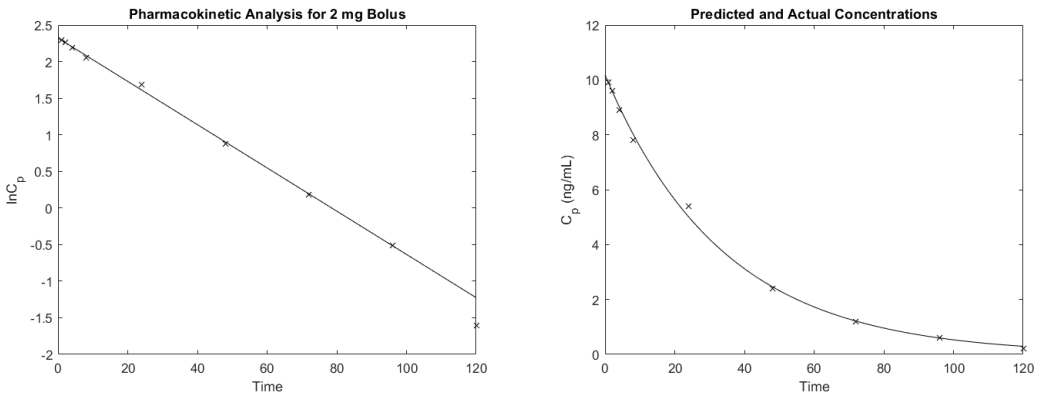
Based on the activity and metabolic stability results of each of the four compounds, the most promising drug was selected for a bolus IV pharmacokinetic study in 8 healthy male volunteers using a dose, $D = 2mg$. We utilize a one-compartment model, consistent with the lack of a noticeable distribution phase on the log-linear plot of mean plasma concentration versus time, to determine the volume of distribution, V_1, elimination rate constant, k_e, and elimination half-life, $t_{1/2}$. The relevant formulae for this model are the one-compartment exponential decay equation and the relationship between rate constant and half-life. The latter is simply $t_{1/2} = \ln 2/k_e$, and the former is given by (in logarithmic terms):

$$\ln C_p = \frac{D}{V_1} - k_e t \tag{2}$$

A linear regression was performed in Matlab to analyze the plasma concentration vs. time data with known dose, revealing parameters $V_1 = 196L$ and $k_e = 0.0295hr^{-1}$. As a result, the elimination half life is $t_{1/2} = 23.5hr$. The predicted results and the experimental observations are displayed on a log-linear and linear-linear scale in Figure 1. The volume of distribution is consistent with rapid movement of drug throughout the body into the periphery owing to its low solubility and indicating the need for a sustained release system, hereafter explored in the form of an injectable polymeric microparticle.

3 Question 3: AUC and Burst of Long-Acting Injectable Antipsychotic Formulations

Rather than continue development of a drug for intravenous administration, owing to the invasive, uncomfortable, and time-consuming nature of such an intervention, development began on a Long Acting Injectable (LAI) Antipsychotic using the above compound. The selected system is an injectable poly(lactide-co-glycolide) (PLGA) depot with a



(a) Log-linear.

(b) Linear.

Figure 1: Linear regression pharmacokinetic analysis using a one-compartment model.

target duration of release of 2 weeks, such that dosing may occur every 2 weeks while plasma levels remain relatively stable and above the threshold for activity. The product should also display minimal lag time upon injection and minimal burst (rapid initial release following injection). Six different formulations were tested at body temperature and neutral pH *in vitro* to examine the burst release characteristics at the 24 hr time point, and an *in vivo* pharmacokinetic study was performed over 35 days with intramuscular injection of $0.5mg/kg$. The six formulations, $A - F$, differ in their molecular weight, ranging from $175kDa$ down to $15kDa$, and lactide-to-glycolide mole ratios of $85 : 15$ down to $50 : 50$.

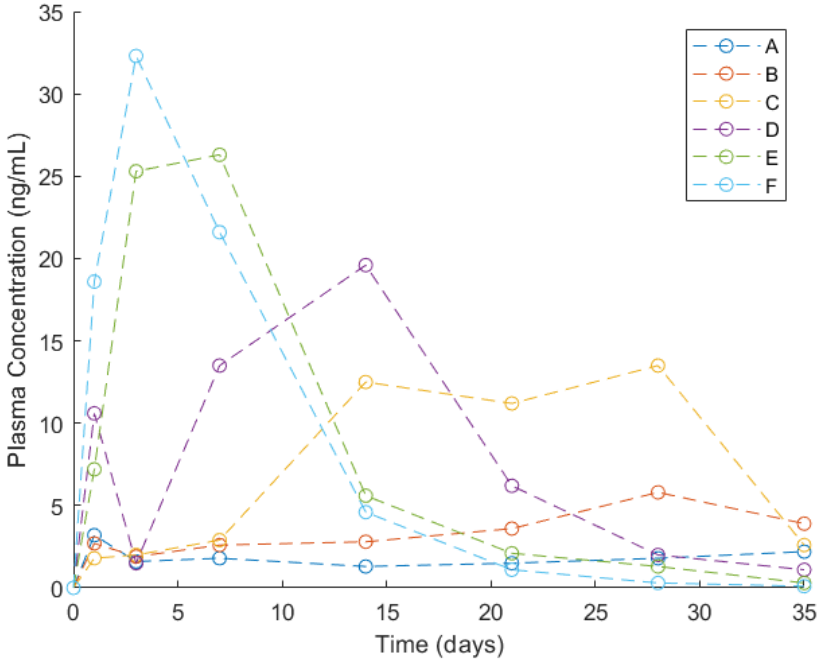


Figure 2: Time course of plasma concentration for each of the six formulations.

To quantify the area under the curve (AUC), a representation of the total delivered drug over the experimental time course, we plot the plasma concentration as a function of time for each of the six formulations (as shown above) and use Matlab's *trapz* function, which approximates the integral of a curve using the trapezoid rule. This simple approximation of an integral takes each of the N data points and linearizes adjacent points, then finds the area of

the trapezoid encompassed by each of the pairs of adjacent points and sums the results. In mathematical terms,

$$\int_a^b f(x)dx \approx \frac{1}{2}\Sigma_{n=1}^{N}(x_{n+1} - x_n)[f(x_{n+1} + f(x_n)] \tag{3}$$

Using this method, we obtain the AUCs shown in Table 2 (absolute, as well as normalized to that of Formulation D). Also included, for reference, is the percentage of total drug load released in the first day (i.e. burst) *in vitro*.

Table 2: AUC of Candidate Formulations.

Compound	**AUC (ng*d/mL)**	**Normalized AUC to D**	**24-hr burst release (%)**
A	59.40	0.203	3
B	123.1	0.420	5
C	294.2	1.00	4
D	293.1	1.00	12
E	295.4	1.01	8
F	286.0	0.976	15

From these results, it is clear that formulations A and B show the lowest AUC, indicative of the lowest amount of drug release over the experiment. This is consistent with what one would expect based on the material properties of the PLGA depot. In both of these formulations, the lactide-to-glycolide ratio is $85:15$. As lactide is a substantially more hydrophobic polymer, owing to the presence of a methyl group, we would expect these two formulations to display more hydrophobic character, and consequently, a much slower degradation rate. Similarly, we also expect a slower rate of infiltration of water, and thus slower swelling and drug diffusion out of the microsphere. In other words, both the initial hydration-induced release and sustained degradation-induced release are expected to slow. This is further supported by the low burst percentages for these two formulations (3 and 5). The larger molecular weight of the first formulation further enhances these properties, as larger chains will need to swell more and be degraded further in order to relax enough to permit drug efflux or to be dissolved into the surrounding solution. The lower composition ratio of the other four formulations provides sufficient hydrophilicity to allow for sustained drug release. In order to minimize burst while maintaining elevated levels over the 35-day window, we would likely select formulation C. As discussed above, the first two formulations do not release large amounts of drug, a conclusion further supported by the low plasma concentrations for these formulations (around $5ng/mL$ or below for the entire experiment). Formulations D, E, and F show large amounts of burst, leading to rapid, sharp peaks in the plasma concentration and falling to low levels within 15-20 days. Formulation C, however, releases a comparable amount of drug to these three, but does so in a more sustained manner after an initial 6-7 day lag phase.

We can also compare the reported *in vitro* burst levels to the burst levels recorded in the *in vivo* rat study, indicated by the plasma concentration of the active agent at the 1-day mark. The figure below plots these two, along with a trend line obtained from a linear regression. Correlation analysis yields a correlation coefficient of 0.965 and a p-value of 0.002, suggesting a statistically significant relationship between the two parameters and supporting the accuracy of the *in vitro* results.

4 Question 4: Pharmacodynamic Analysis of Mean D2 Receptor Occupancy

As a means to assess the physiological efficacy of the chosen drug formulation, positron emission spectroscopy was employed to quantify the percentage of D2 receptors occupied by the drug as a function of plasma concentration in 14 male schizophrenic patients. Doses were varied to yield a range of steady-state plasma concentrations at the end of a six-day period, with infusions occuring daily by intravenous administration. By fitting the resultant plasma concentration and D2 receptor occupancy percentage to an E_{max} pharmacodynamic model, using Matlab's $fitnlm$ function, with baseline occupancy $E_0 = 0\%$, maximum occupancy $E_{max} = 100\%$, and a slope factor $n = 1$, consistent with other atypical antipsychotics, we obtain a concentration for half-maximal effect, $EC_{50} = 2.18ng/mL$. The E_{max} model is defined by the equation $E = E_0 + E_{max}\frac{C^n}{EC_{50}^n + C^n}$, which relates the effect, E, to the concentration of the active substance, C.

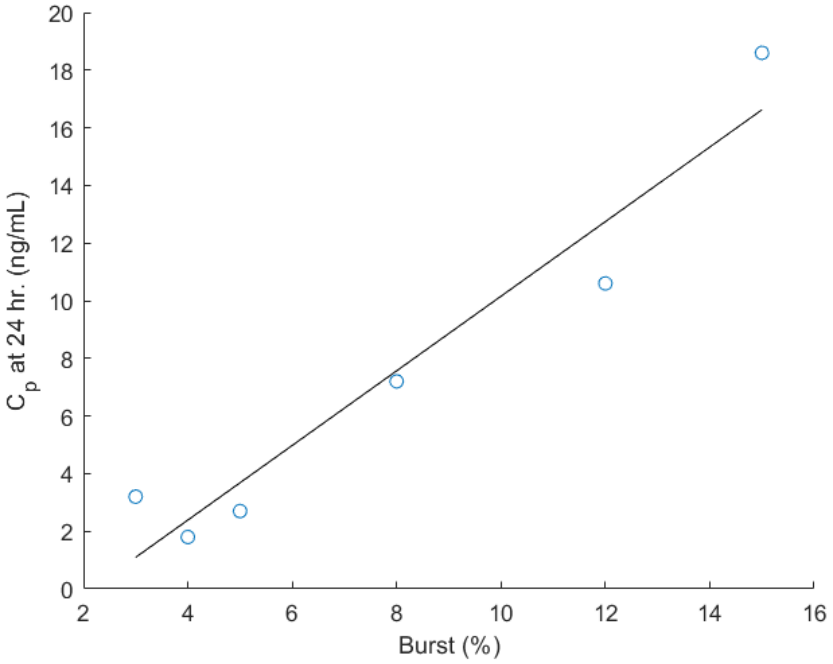


Figure 3: Correlation between *in vitro* burst and day one plasma concentration in rats.

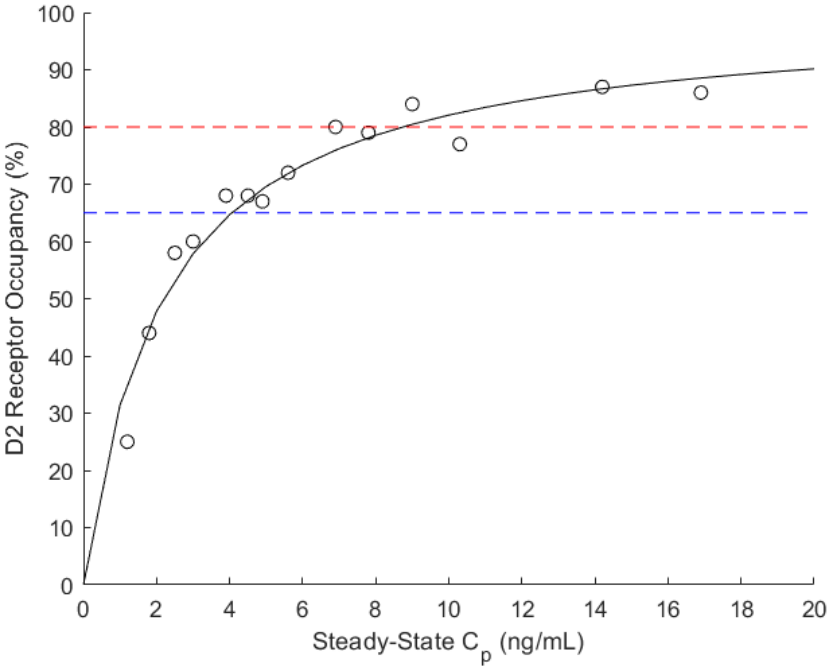


Figure 4: Pharmacodynamic E_{max} model for D2 receptor occupancy.

The red and blue horizontal lines on the above plot denote the threshold for adverse side effects, 80%, and threshold of efficacy, 65%, respectively. Though it was found that no side effects arise even at 87% occupancy, we utilize the previously reported upper threshold as a precaution. To target the center of this desired range, we would like a plasma concentration of approximately $6ng/mL$, which should yield an occupancy around 73%, though anything ranging from $4ng/mL$ to $9ng/mL$ should suffice, and even up to $13ng/mL$ would be acceptable for the 87% upper bound.

5 Question 5: Bioavailability of Long-Acting Injectable Antipsychotic and Plasma Concentration Interpolation

Results from a phase I clinical trial with six schizophrenic partients in response to a 35 mg dose of the LAI over 28 days (672 hours) were obtained. We can quantify the AUC as previously done for the preclinical formulation study, and compare it to the AUC for the intravenous administration. The latter quantity can be found by integrating the one-compartment model fit for the pharmacokinetic analysis in Question 2 above, after extrapolating the curve to the same time point (672 hours). Given that this equation is a simple exponential, the area under the curve (dividing out by the dose to allow for comparison of the two quantities) is then:

$$AUC_{IV} = \int_0^{672} C_p(t)dt = \frac{1}{V_1}\int_0^{672} e^{-k_e t}dt = \frac{-1}{k_e V_1}[e^{-672k_e} - 1] \tag{4}$$

This expression gives us an area under the curve of $1.722 * 10^{-4} hr/mL$. As a form of sanity check, we can compute the same integral using the *trapz* function in Matlab on the concentration profile predicted by the one-compartment model rather than computing the integral manually. This yields an AUC of $1.723 * 10^{-4}$, nearly equal aside from some integration error imparted by the trapezoid rule. As a final sanity check, we can also compare the predicted AUC from the model up to 120 hr to the true data used to fit the model parameters. These calculations yield AUCs of $1.67 * 10^{-4}$, confirming the model accuracy.

The AUC of the LAI administration is calculated to be $1.093 * 10^{-4} hr/mL$ after normalization by the dose. We can estimate the bioavailability by dividing these two AUC quantities after normalizing each by the administered dose (2 mg and 35 mg for IV and LAI, respectively). This calculation yields a bioavailability of 63.5%. In order to predict

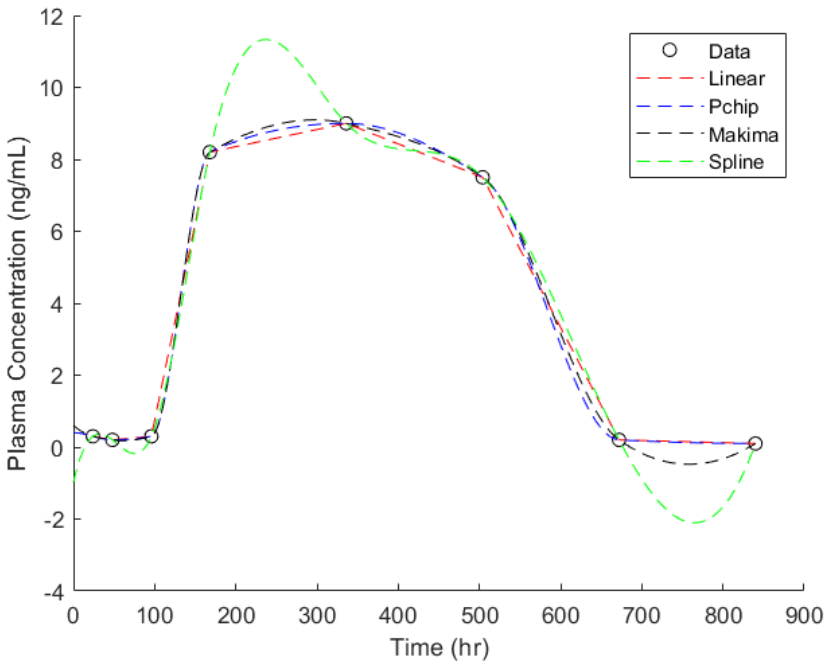
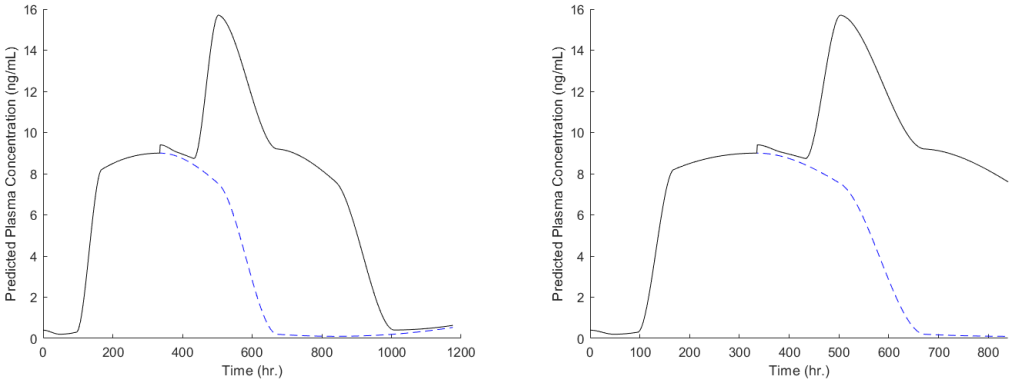


Figure 5: Interpolation Methods for Long-Acting Injectable Dosage.

plasma concentration following multiple dose events, we employ the principle of superposition, assuming that the plasma concentration change from each dose event is independent of the other events, so the plasma concentration at a given time point will be the sum of the contribution of each dose. Therefore, we need to interpolate between the experimental data points over the length of the experimental time window. We employ four different interpolation schemes available in Matlab's *interp*1 function - linear, shape-preserving piecewise cubic (pchip), modified Akima cubic Hermite, and cubic spline. The Akima algorithm produces piecewise polynomials with continuous first-order derivatives to preserve slope and reduce fluctuations, with a modification that more weight is given to the smaller slope if two flat regions meet. In contrast, the spline algorithm produces piecewise polynomials with continuous

second-order derivatives. Clearly, even though this final method is reported to reduce heavy oscillations when compared to typical polynomial interpolation methods, it is still subject to large oscillations with the data used here, most notably after the steepest rise and fall in plasma concentration. Linear interpolation is not continuous in the derivative, and thus is unlikely to provide a useful estimate of plasma concentration. The modified Akima interpolation too experiences some degree of overshoot and undershoot around the same transition points as the cubic spline. The remaining method, pchip or piecewise cubic Hermite interpolating polynomial, does not require a continuous second derivative, leading to a less smooth interpolation for functions which exhibit large oscillations. For our plasma concentration data, however, the result is a smooth profile over time that does not cross the zero concentration level and exhibits a gradual return to baseline after around 700 hours. As a result, we select the pchip profile for subsequent superposition.



(a) Total. (b) Truncated.

Figure 6: Superposition of Plasma Concentration Profiles for Doses at $t = 0hr$ and $t = 336hr$.

To superpose the influences of two discrete dose administrations, we consider the profile between time 0 and time $t_2 + 840hr$, as $840hr$ is the furthest data point at which we have data. The interpolation function is evaluated in increments of one hour up until this final time point. For the dose administered at $t_1 = 0hr$, the profile is simply this interpolation function. For the dose administered at time t_2, here set to be $336hr$, we set the first t_2 time points to be $C_p = 0ng/mL$ and then take the next 840 time points to be the interpolation function. It should be noted that this requires the interpolation function for the first profile to function as an extrapolation function beyond the $840hr$ data point. The interpolation function, shown as the blue dotted line, clearly begins to be inaccurate beyond the limits of the experimental data, as it predicts the concentration of drug in the plasma will begin to gradually increase. Therefore, it is more reliable to truncate the interpolation at time $840hr$, since this is the only region in which the interpolation function was fit.

In either case, the results are similar - the elevated region of the plasma concentration from a single dose occurs from around $150hr$ through $550hr$ after administration, with the peak around the time of the second dose ($336hr$). Given the prolonged length of this elevated region, administering the second dose at the maximal time of the first produces a sharp spike resulting from elevated concentrations produced by both LAI doses. This concentration peaks at almost $16ng/mL$, nearly double that of a single dose. The influence of the first dose then falls sharply, and the second injection dominates the concentration until it wears off around $1000hr$. At a time of $504hr$, this method predicts a plasma concentration $C_p(504) = 15.7ng/mL$, which, using the previously derived E_{max} model, corresponds to a D2 receptor occupancy of 87.8%, above any of the thresholds for adverse side effects. Judging by these results, it would be prudent to deliver the second dose later. For example, the final figure shows the same simulation with a second dose administration occurring at $460hr$. Unfortunately, a preliminary analysis does not reveal a simple way to combine two doses such that the fluctuation in plasma concentration does not occur, but using this time point predicts the maximal plasma concentration will not exceed $10ng/mL$, and thus the maximum receptor occupancy should not exceed 82%. Additionally, in the transition region, the minimal concentration is around $5.6ng/mL$, corresponding to a receptor occupancy of 72%, well above the desired level for therapeutic effect.

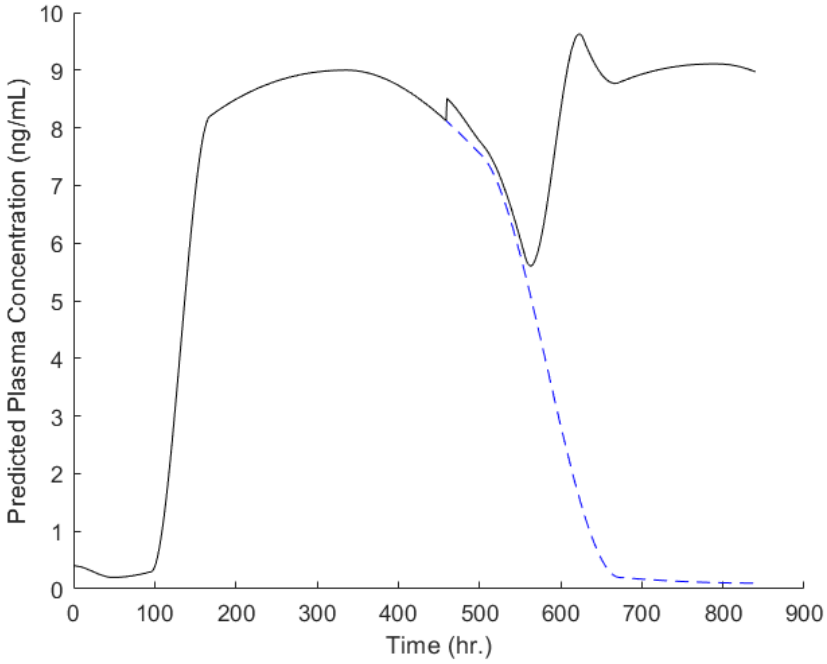


Figure 7: Superposition of Plasma Concentration Profiles for Doses at $t = 0hr$ and $t = 460hr$.

6 Appendix

Script to calculate solubility as a function of pH for four tertiary amines based on pKa and equilibrium between protonated and deprotonated amine groups.

```
MW = [422,394,388,465];
So = [11.2,4.6,2.9,5.8];
pka = [8.6,9.1,9.3,8.6];
ka = 10.^-(pka);

pH = [5,6,7];
H = 10.^-(pH);

Sw = zeros(length(pH),length(So));

for i = 1:length(pH)
    Sw(i,:) = So.*(1+H(i)./ka);
end
```

Script to determine pharmacokinetic parameters for a one-compartment model with a dose of 2000000 ng.

```
t = [1,2,4,8,24,48,72,96,120];
Cp = [9.9,9.6,8.9,7.8,5.4,2.4,1.2,0.6,0.2];
D = 2000000;

figure;
plot(t,log(Cp),'xk','LineStyle','none');
xlabel('Time');
ylabel(['lnC_p']);
title('Pharmacokinetic Analysis for 2 mg Bolus');
hold on;

[fit,p] = polyfit(t(1:end-1),log(Cp(1:end-1)),1);
```

```
time = [0:0.5:120];
modellogCp = fit(1)*time+fit(2);
SSE = 0;
for i = 1:length(Cp)-1
    SSE = SSE + (fit(1)*t(i)+fit(2)-log(Cp(i)))^2;
end
plot(time, modellogCp,'k');
hold off;
V1 = D/exp(fit(2));
ke = -fit(1);
t12 = log(2)/ke;

figure;
plot(t,Cp,'xk','LineStyle','none');
hold on;
modelCp = (D/V1)*exp(-ke*time);
plot(time,modelCp,'k');
xlabel('Time');
ylabel(['C_p (ng/mL)']);
title('Predicted and Actual Concentrations');
```

Script to calculate area under the curve (AUC) of plasma concentrations over time based on Matlab's trapz function. This also plots the temporal profile of each of the six formulations, normalizes the AUC by one of the formulations (d) to facilitate comparison, and calculates and plots the correlation between in vitro burst after 24 hours and the day one plasma concentration observed in an in vivo rat model.

```
t = [0,1,3,7,14,21,28,35];
Cp = [0,3.2,1.6,1.8,1.3,1.5,1.8,2.2;
    0,2.7,1.9,2.6,2.8,3.6,5.8,3.9;
    0,1.8,2.0,2.9,12.5,11.2,13.5,2.6;
    0,10.6,1.5,13.5,19.6,6.2,2.0,1.1;
    0,7.2,25.3,26.3,5.6,2.1,1.3,0.3;
    0,18.6,32.3,21.6,4.6,1.1,0.3,0.1];
numform = size(Cp,1);
AUC = zeros(size(Cp,1),1);
burst = [3,5,4,12,8,15];
figure;
hold on;
for i = 1:numform
    plot(t,Cp(i,:),'--o');
    AUC(i) = trapz(t,Cp(i,:));
end
AUCd = AUC(4);
AUCnorm = AUC/AUCd;
xlabel('Time (days)');
ylabel('Plasma Concentration (ng/mL)');
legend(['A';'B';'C';'D';'E';'F']);
hold off;

figure;
hold on;
plot(burst',Cp(:,2),'o','LineStyle','none');
xlabel('Burst (%)');
ylabel('C_p at 24 hr. (ng/mL)');
[fit,p] = polyfit(burst',Cp(:,2),1);
b = [3:1:15];
C = fit(1)*b+fit(2);
plot(b,C,'k');
```

```
[burstcorr,pval] = corr(burst',Cp(:,2));
```

Script for fitting the pharmacodynamics between plasma concentration of the tertiary amine LAI formulation and D2 receptor occupancy. The only parameter to be fit in this model is the concentration for half-maximal effect, EC50. The model is fit using nonlinear regression. Also plotted are the thresholds for efficacy and for adverse side effects.

```
Emax = 100;
n = 1;
Cp = [1.2,1.8,2.5,3,3.9,4.5,4.9,5.6,6.9,7.8,9,10.3,14.2,16.9];
D2 = [25,44,58,60,68,68,67,72,80,79,84,77,87,86];

figure;
hold on;
plot(Cp,D2,'ok','LineStyle','none');
xlabel('Steady-State C_p (ng/mL)');
ylabel('D2 Receptor Occupancy (%)');

E = @(coeff,C) Emax*C./(coeff+C);
cf = fitnlm(Cp,D2,E,2);

EC50 = cf.Coefficients.Estimate;

Cpmodel = [0:20];
D2model = E(EC50,Cpmodel);
plot(Cpmodel,D2model,'k');

bound1 = 65*ones(21,1);
bound2 = 80*ones(21,1);
plot(Cpmodel,bound1,'--b');
plot(Cpmodel,bound2,'--r');

hold off;
```

Script for determining bioavailability of the LAI formulation by finding AUC from the LAI data using trapz and from integrating the data predicted by the pharmacokinetic model for IV administration. The LAI plasma concentration profile is then interpolated using four different 1D interpolation methods, and the best method (pchip) is used to superpose the profiles of two LAI doses by shifting the later dose and concatenating the vector with preceding zeros. The concentration at the specified time point (504) is then extracted and the previous pharmacodynamic model is used to predict the receptor occupancy.

```
t = [24,48,96,168,336,504,672,840];
Cp = [0.3,0.2,0.3,8.2,9.0,7.5,0.2,0.1];
AUCLAI = trapz(t,Cp);
AUCLAImg = AUCLAI/35000000;
time = 0:840;
CpIV = (1/V1)*exp(-ke*time(1:673));
AUCIVmg = trapz(CpIV);

F = 100*AUCLAImg/AUCIVmg;
figure;
hold on;
plot(t,Cp,'-ok','LineStyle','none');
xlabel('Time (hr)');
ylabel('Plasma Concentration (ng/mL)');

%%%Interpolation stuff%%%
I1 = interp1(t,Cp,time,'linear');
I2 = interp1(t,Cp,time,'pchip');
```

```
I3 = interp1(t,Cp,time,'makima');
I4 = interp1(t,Cp,time,'spline');
plot(time,I1,'--r');
plot(time,I2,'--b');
plot(time,I3,'--k');
plot(time,I4,'--g');
%%%

legend({'Data','Linear','Pchip','Makima','Spline'});
hold off;

t1 = 0;
t2 = 336;
ttot = 0:(840+t2);
Cpinter = interp1(t,Cp,ttot,'pchip');
Cp1 = [zeros(1,t1),Cpinter(1:ttot(end)-t1+1)];
Cp2 = [zeros(1,t2),Cpinter(1:ttot(end)-t2+1)];
Cptot = Cp1+Cp2;
figure;
hold on;
plot(ttot,Cpinter,'--b');
plot(ttot,Cptot,'k');
xlabel('Time (hr.)');
ylabel('Predicted Plasma Concentration (ng/mL)');
hold off;
Cp504 = Cptot(505);
D2Occ = E(EC50,Cp504);
```